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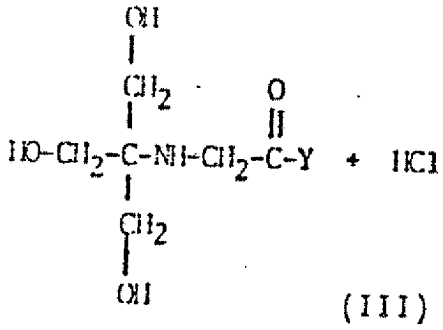
(54) Synthese von Tris-hydroxymethyl-methan substituierten Peptiden.

(57) Die Erfindung betrifft eine Synthese von Tris-hydroxymethylmethan substituierten niederen Peptiden, die durch diese Substitution eine ausgezeichnete Wasserlöslichkeit aufweisen und als Puffer mit weitgespanntem pH-Bereich verwendet werden können, wenn das Peptid saure, neutrale oder/und basische Aminosäuren enthält.

Bestimmte substituierte Peptide dieser Verbindungsklasse erweisen sich als ideale untoxische Puffersubstanzen für die Züchtung tierischer oder menschlicher primärer oder kontinuierlicher Zellinien.



(III)

wobei X = Cl, Br, J und Y eine α–δ- oder α–l-Aminosäure, Di-, Tri- oder Tetrapeptid, bestehend aus β-, γ- oder δ-Aminosäuren und deren Gemische, darstellen.

Die bei der Reaktion freiwerdende Salzsäure wird dadurch abgefangen, indem man für die Reaktion die Alkalisalze der Aminosäuren oder Peptide verwendet. Nach der Reaktion entstehen somit die inneren Salze der Tris-substituierten Aminosäuren und die entsprechenden Salze der Alkali- und Halogengruppe. Die Reaktionen werden potentiometrisch verfolgt. Nach pH-Konstanz ist die Umsetzung vollständig.

Nimmt man zur Synthese N-2-halogenacetylierte α-l-Aminosäuren, so werden Puffersubstanzen erhalten, welche für primäre und kontinuierliche Zellinien in Konzentrationen bis zu 0,1 M absolut untoxisch sind.

Beispiel 1

Zu einer gesättigten, wässrigen Lösung von 1 Mol N-2-chloracetyl-1-methionin (Na-Salz) gibt man 1,05 Mol Tris-hydroxymethyl-aminomethan und erwärmt das Gemisch zum Siedepunkt unter Rückfluss für vier Stunden. Nach Zusatz von Äthanol bis zum Trübungspunkt in der Hitze lässt man das Produkt (Tris-Gly-met) unter Abkühlen auskristallisieren.

Beispiel 2

Zu einer gesättigten, wässrigen Lösung von 1 Mol N-2-J-acetyl-1-tyrosin (Li-Salz) gibt man 1,05 Mol Tris-hydroxymethyl-aminomethan und erwärmt das Gemisch zum Siedepunkt unter Rückfluss und Lichtausschluss für 15 Minuten. Nach Zusatz von Äthanol bis zum Trübungspunkt in der Hitze lässt man das Produkt (Tris-Gly-Tyr) unter Abkühlen auskristallisieren.

Beispiel 3

Zu einer gesättigten, wässrigen Lösung von 0,1 Mol N-2-Br-acetyl-1-gly-1-arg-d-asp-1-threo (K-Salz) gibt man 0,1 Mol Tris-hydroxymethyl-aminomethan und erwärmt das Gemisch zum Siedepunkt unter Rückfluss und Lichtausschluss für 45 Minuten. Nach Zusatz von Isopropanol bis zum Trübungspunkt in der Hitze lässt man das Produkt (Tris-Gly-Gly-arg-ser-threo) unter Abkühlen auskristallisieren.

Beispiel 4

Zu einer gesättigten, wässrigen Lösung von 0,05 Mol N-2-chloracetyl-γ-aminobuttersäure (K-Salz) gibt man 0,05 Mol Tris-hydroxymethyl-aminomethan und erwärmt das Gemisch zum Siedepunkt unter Rückfluss für fünf Stunden. Nach Zusatz von Propanol bis zum Trübungspunkt in der Hitze lässt man das Produkt (Tris-Gly-γ-butyr) unter Abkühlen auskristallisieren.

translation of Swiss patent CH 640 511

Swiss Patent 640 511

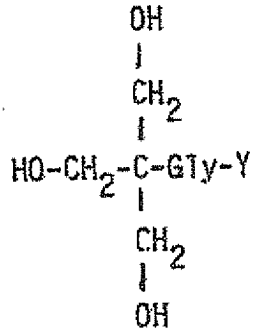
Synthesis of trishydroxymethylmethane-substituted peptides.

The invention relates to a synthesis of trishydroxymethylmethane-substituted lower peptides which have, owing their substitution, an excellent solubility in water and can be used as buffers with a wide pH range when the peptide comprises acidic, neutral or/and basic amino acids.

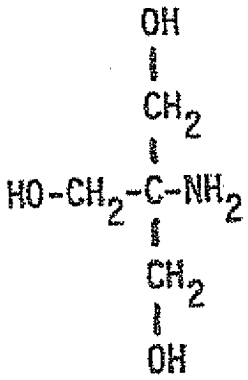
Certain substituted peptides of this class of compounds prove to be ideal non-toxic buffer substances for culturing animal or human primary or continuous cell lines.

CLAIMS

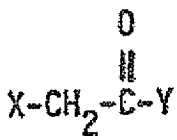
1. Synthesis of trishydroxymethylmethane-substituted lower peptides of the formula



characterized in that trishydroxymethylaminomethane



is reacted with N-(2-haloacetylated) amino acids or N-(2-haloacetylated) di-, tri- and tetrapeptides of the formula



where X is Cl, Br or I and Y is an amino acid or a di-, tri- or tetrapeptide.

2. Synthesis according to Claim 1, characterized in that the amino acid represents an α-l- or α-d-amino acid.

3. Synthesis according to Claim 1, characterized in that the di-, tri- or tetrapeptide consists of β-, γ- or δ-amino acids or mixtures thereof.

4. Synthesis according to Claim 1, characterized in that the di-, tri- and tetrapeptides according to Claim 3 comprise acidic and basic amino acids, for example amino dicarboxylic acids and diamino carboxylic acids, in the molecule.

5. Synthesis according to Claim 1-4, characterized in that the alkali metal salts of the amino acids or di-, tri- and tetrapeptides are used.

6. Use of the peptides obtained according to Claim 1 and 2 as non-toxic buffers for all biological systems, where all natural l-amino acids stand for the amino acid.

7. Use according to Claim 6, characterized in that Tris-Gly-Ala is employed as non-toxic buffer substance for continuous and primary cell lines.

8. Use according to Claim 6, characterized in that Tris-Gly-Met is employed as non-toxic buffer substance for continuous and primary cell lines.

In vitro cultures of animal and human primary or continuous (transformed) cell lines represent extremely sensitive biological systems. The cells must for optimal proliferation thereof, besides a constant temperature, addition of serum and complicated nutrient solutions, in particular be efficiently buffered around the physiological pH (7.2-7.4) with non-toxic substances.

Whereas synthetic buffer substances such as piperazine-sulphonic acid derivatives have proved to be non-toxic for a few continuous cell lines, although only in low concentrations (20-50 mM), the culturing of most cell line depends on the $CO_2/NaHCO_3$ buffer system. This has the disadvantage that operation in open vessels or systems is necessary in order to ensure gas exchange. Contamination by bacteria, mycoplasmas, yeast and fungi can be reduced to a minimum only by employing particular compounds (antibiotics, fungicides), although these compounds impair the growth of animal or human cells. Anticontamination agents are particularly undesirable when human vaccines need to be prepared on human primary cell lines.

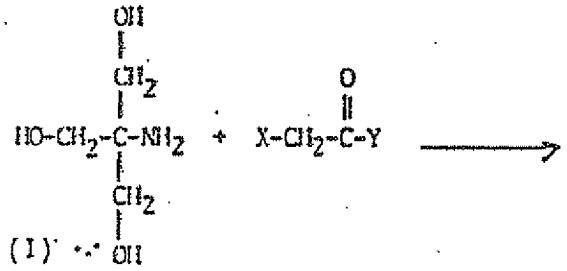
These sterility problems and the use of antibiotics can be completely eliminated if it is possible to culture

primary human cells in closed systems, this requiring the use of non-toxic buffers.

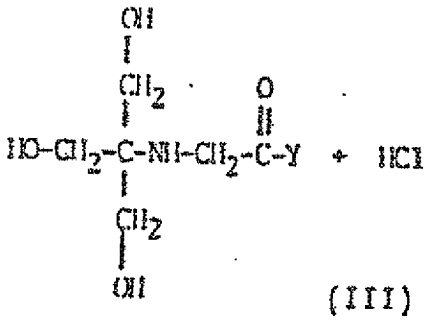
It has emerged that lower peptides or trishydroxymethyl-methane-substituted lower peptides are very suitable as non-toxic buffers for this purpose. Lower peptides have a good buffer capacity in a wide pH range (depending on the amino acid composition), but virtually no consideration is given to their use as buffer substances because their solubilities in protic solvents are too low and are often metabolized, that is to say consumed, by biological systems.

Both disadvantages can be eliminated by suitable substituents on the peptides. The trishydroxymethyl-methane residue has as substituent for this purpose the necessary molecular properties of converting lower peptides into a readily water-soluble form. This takes place by reacting trishydroxymethylaminomethane with N-2-haloacetylated amino acids or N-2-haloacetylated di-, tri- and tetrapeptides in aqueous solution.

In this condensation reaction, the amino acid or the lower peptide is extended at the N terminus by one glycine residue which is in turn substituted by trishydroxymethylmethane. It is possible thereby to synthesize trishydroxymethylmethane-substituted peptides very generally, as follows:



(I) (II)



(III)

where X is Cl, Br, I and Y is an α-δ- or α-l-amino acid, di-, tri- or tetrapeptide consisting of β-, γ- or δ-amino acids and mixtures thereof.

The hydrochloric acid liberated in the reaction is trapped by using the alkali metal salts of the amino acids or peptides for the reaction. Thus, the reaction results in the inner salts of the tris-substituted amino acids and the corresponding salts of the alkali metal and halogen groups. The reactions are followed by potentiometry. The reaction is complete when the pH is constant.

Using N-2-haloacetylated α-l-amino acids for the synthesis results in buffer substances which are absolutely non-toxic for primary and continuous cell lines in concentrations up to 0.1 M.

Example 1

1.05 mol of trishydroxymethylaminomethane are added to a saturated aqueous solution of 1 mol of N-2-chloroacetyl-l-methionine (Na salt), and the mixture is heated to boiling under reflux for four hours. After addition of ethanol to the hot mixture until it is cloudy, the product (Tris-Gly-met) can be crystallized by cooling.

Example 2

1.05 mol of trishydroxymethylaminomethane are added to a saturated aqueous solution of 1 mol of N-2-I-acetyl-l-tyrosine (Li salt), and the mixture is heated to boiling under reflux with exclusion of light for 15 minutes. After addition of ethanol to the hot mixture until it is cloudy, the product (Tris-Gly-Tyr) can be crystallized by cooling.

Example 3

0.1 mol of trishydroxymethylaminomethane is added to a saturated aqueous solution of 0.1 mol of N-2-Br-acetyl-l-gly-l-arg-d-asp-l-threo (K salt), and the mixture is heated to boiling under reflux with exclusion of light for 45 minutes. After addition of isopropanol to the hot mixture until it is cloudy, the product (Tris-Gly-Gly-arg-ser-threo) can be crystallized by cooling.

Example 4

0.05 mol of trishydroxymethylaminomethane is added to a saturated aqueous solution of 0.05 mol of N-2-chloroacetyl-γ-aminobutyric acid (K salt), and the mixture is heated to boiling under reflux for five hours. After addition of propanol to the hot mixture until it is cloudy, the product (Tris-Gly-γ-butyr) can be crystallized by cooling.